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Artigo

Cissampelos sympodialis Eichl. (Menispermaceae) inhibits anaphylactic shock reaction in murine allergic model

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ABSTRACT: The murine model of OVA-induced immediate allergic reaction was used to evaluate the effectiveness of intraperitoneal sub-acute treatment with the leaf hydroalcoholic extract of *Cissampelos sympodialis* (AFL) in the anaphylactic shock reaction, IgE production and the background proliferative response. BALB/c mice treated with AFL ranging from 200 to 400 mg/kg/day for 5 days before and during OVA-sensitization strongly reduced the animal death and promoted reduction in total and OVA-specific serum IgE level. Spleen cells from AFL-treated sensitized animals showed a decreased proliferative background response when compared with non-sensitized animals. These results demonstrated that sub-acute intraperitoneal treatment with *Cissampelos sympodialis* extract has an anti-allergic effect.

Keywords: *Cissampelos sympodialis*, BALB/c mice, sub-acute treatment, anaphylactic shock reaction, Immunoglobulin E.

INTRODUCTION

Anaphylactic shock reaction is a serious and potentially lethal systemic reaction caused by immediate allergic response (Moneret-Vautrin et al., 2005). It is well described that food, drugs and insect bits might evoke fatal anaphylaxis in sensitized subjects, and the number of death related to allergic response has increased in the last years (Scott; Donald, 2004).

New drug researches to allergic responses have indicated that medicinal plants consist on important tools to treat immediate allergic reactions (Dai et al., 2004). In accordance with this perspective, we studied a potential effect of a native Brazilian plant named *Cissampelos sympodialis* in immediate allergic response.

The genus *Cissampelos* is known by the presence of alkaloids (Barbosa-Filho et al., 1997) and some of these molecules have been isolated from *C. sympodialis* as milonine, laurifoline, roraimine, warifteine and methylwarifteine which has spasmolytic action (Cortes et al., 1995; Freitas et al., 1996).

In vitro immunological studies showed that the leaf hydroalcoholic extract of *Cissampelos sympodialis* (AFL) increased the production of interleukin-10 (IL-10) and inhibited T cell proliferative response in mouse spleen cells stimulated with Concanavalin-A (Piuvezam et al., 1999). AFL also induced an IL-10-dependent inhibition of *Trypanosoma cruzi* killing by macrophages and modulated B cell response (Alexandre-Moreira et al., 2003a,b). Studies *in vivo* demonstrated that intraperitoneal injection of AFL has an anti-inflammatory activity in the carrageenan or capsaicin mouse ear edema (Batista-Lima et al., 2001).

In addition, Bezerra-Santos (2004) showed that oral treatment with AFL reduced not only total and OVA-specific IgE level but also induced INF-γ production in sensitized BALB/c mice. Taking together both *in vitro* and *in vivo* studies described above, it can be suggested that *Cissampelos sympodialis* has an important immunopharmacological activity.

Some types of immediate allergic reactions are now considered to be a disorder associated with increased of IgE levels (Mayr; Zuberi; Liu, 2003). IgE responses might be lethal due to mediators delivering such as histamine from mast cell sensitized with IgE and consequent anaphylactic shock reaction (Reviewed by Finkelman et al., 2005; Strait et al., 2002).

Compound 48/80 causes peritoneal mast cell degranulation and respective mediator releases (Kim et al., 2000). Indeed, this molecule seems activate mast cell directly without specific receptor differently IgE responses (Ferry et al, 2002). Studies using compound 48/80 have allowed understanding some plant mechanisms in order to prevent allergic diseases (Dai et al., 2004; Phil-Dong et al., 2004).

To amplify our knowledge on the suitability to use the *Cissampelos sympodialis* for the treatment of allergic diseases we evaluated in this study the effect of the intraperitoneal treatment with AFL in ovalbumin-sensitized BALB/c mice. The immunological aspects investigated here were the anaphylactic shock reaction induced by ovalbumin or compound 48/80,

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Table 1. Effect of AFL in anaphylactic shock reaction

Treatment	Dose (mg/kg)	Ovalbumin	Mortality %	Compound 48/80	Mortality %
Saline	-	+	100	+	100
AFL	200	+	50*	+	100
	400	+	30*	+	100

Groups of mice (n=10) received AFL sub-acute treatment before and during OVA sensitization protocol. Three weeks after the first OVA injection, the animals received OVA challenged by i.v. route. In a separated experiment, mice were treated with AFL 1 h before compound 48/80 injection. The mortality was monitored 1h after OVA or compound 48/80 challenges. Negative control group received only saline during experimental protocols and did not present any death response. * $p < 0.001$ compared with control group (OVA) and analyzed by the Fisher's test.

immunoglobulin E production and cell proliferative response.

MATERIAL AND METHODS

Plant material and extract preparation

Leaves from *Cissampelos sympodialis* were obtained from the Botanical Garden of the Laboratório de Tecnologia Farmacêutica/ Universidade Federal da Paraíba (voucher specimen Agra 1456). The leaves were dried at 50 ºC in an oven and pulverized. The powder was extracted with 70% ethanol in water at 70 ºC for 5 days. The dried extract, herein named AFL, was dissolved in water, filtered and known volumes were dried to determine the final concentration of the water-soluble components. All doses are expressed in terms of the concentration of the soluble components (mg/kg of body weight). The yield was 22% on average, based on solid residues present (Thomas et al., 1997).

Animals

BALB/c mice (25-30 g) and Wistar rats (250-300 g) were used throughout the study. The animals were supplied by the Animal Facility from the Laboratório de Tecnologia Farmacêutica (Universidade Federal da Paraíba). Groups of 10 animals were used in each test. The experimental protocols were approved by the Institutional Review Committee for the use of animal subject (CEPA Nº 0602/05) of the Federal University of Paraiba, Brazil. All experiments were carried out with strict adherence to ethical guidelines (Sherwin et al., 2003).

AFL sub-acute treatment and OVA-sensitization

Mice (n=10) were treated with AFL (200 or 400 mg/kg) dissolved in saline for 5 days before and during the sensitization protocol. On day 0 and 14th the mice were actively immunized with OVA (grade II Sigma Chemical, St. Louis, MO) by injection (i.p) of 10 μg OVA adsorbed to 2.25 mg of alum adjuvant. The control group (OVA) received only saline during the OVA immunization (Oshiba et al., 1996). Three weeks after the first OVA injection, animals were exposed to aerosol of 1% (w/v) OVA in saline for 30 min (Randolph et al., 1999).

Anaphylactic shock reaction

In a separated experiment, twenty-three days after initiation of OVA sensitization, AFL-treated sensitized mice were injected intravenously (i.v.) with 200 μl 0.5% Evans Blue solution containing 500 μg OVA (Grunewald et al., 1998). Non sensitized mice were injected with 8 mg/kg of compound 48/80 (Sigma) (Kim et al., 2000). Mortality was monitored for 1 h after induction of anaphylactic reaction.

Determination of OVA-specific antibody by the PCA test

IgE antibody titers were determined using the passive cutaneous anaphylaxis (PCA) reaction. Serum dilutions were inoculated intradermally on the shave backs of Wistar rats. After 24 h the rats received intravenous injection of OVA (2.0mg/rat) in 1% Evans Blue solution. Thirty min later the rats were euthanized and OVA-specific IgE titers were measured. The highest serum dilution giving a 5-mm diameter flare or bluing reaction was taken as the PCA titer (Holt et al., 1981).

Measurement of OVA-specific and total IgE by ELISA

Total or OVA-specific IgE from sera were measured using sandwich ELISA (Snaper; Paul, 1987). Appropriate standards, monoclonal antibodies and alkaline phosphatase-streptavidin (Pharmigen, San Diego, CA) were used as detection reagents. The calibration curve was obtained using purified IgE monoclonal antibody (Pharmigen, San Diego, CA).

Cell preparation and proliferation assay

Spleen cell suspensions were prepared and depleted of red blood cells by incubation in Boyle's solution (0.17 M Tris, 0.16 M ammonium chloride, BDH, UK). The cells were washed twice in RPMI medium (Gibco, Pisley, UK) by centrifugation at 200x g for 10 min. The obtained pellet was resuspended in complete RPMI medium (10% fetal bovine serum). Viable cell number was determined by Trypan Blue exclusion. Triplicate cultures were performed in 96-well flat-bottomed tissue culture plates (Costar, Cambridge, MA, USA) in a final volume of 200 µl per well containing 4-5 x 10^5 cells. Cultures were then incubated for 3 days at 37ºC, 5% CO_2. Proliferation assay was performed as described by Mosmann (1983).

Statistical analyses

Data were statistically analyzed by the Student's t-test using a computer program GraphPad Prism version 3.02. Data were expressed as a mean ± SEM. Results with $p < 0.05$ were considered statistically significant.

RESULTS

Effect of AFL on anaphylactic shock reaction

Sub-acute AFL-treatment inhibited the animal rate mortality by OVA challenge at 50% (200 mg/kg) and 30% (400 mg/kg). However AFL-treatment did not inhibit the anaphylactic shock reaction induced by compound 48/80 injection (Table 1).

Effect of AFL on OVA-specific-IgE and total antibody production

We next investigated the ability of AFL to modify antigen-induced serum IgE production. Table 2 shows the AFL-inhibitory effect on antigen-induced IgE production. OVA sensitization induced an OVA-specific-IgE titer of 1:1.373 (100%), whereas intraperitoneal administration of AFL at 200 or 400mg/kg during the OVA sensitization period reduced the IgE titers by 1:588 (47%) and 1:432 (31%), respectively (Table 2). Similar but less striking results were obtained using the PCA assay (Table 2). We also observed a significant decrease in total antibody IgE levels in the sera of AFL-treated mice.

Effect of AFL on background proliferative response

Background of the cell proliferative response was inhibited, in a dose dependent manner, in AFL-treated mice at doses of AFL 200 mg/kg or 400 mg/kg (0.4 ± 0.03 or 0.15 ± 0.01, $p < 0.05$) when compared with non-treated mice (0.5 ± 0.02), respectively (Figure 1).

DISCUSSION AND CONCLUSION

In this study, we demonstrated that intraperitoneal sub-acute treatment with AFL inhibited the anaphylactic shock reaction in OVA sensitized-BALB/c mice. The anaphylactic shock reaction may be lethal in atopic subjects and it is the most important symptom in hypersensitivity reaction type I (Finkelman et al., 2005; Strait et al., 2002). It has been described that plant extract used in folk Chinese medicine were capable to block shock responses and this effect was related with decreased IgE levels (Hyung-Min et al., 2001). Our study showed similar pattern, since the inhibition of the shock response in AFL treated mice was

Table 2. Serum levels of IgE in AFL treated and OVA-immunized mice

Treatments[a]	Total serum IgE concentration (µg/ml) %	OVA-specific IgE serum titer (%)	OVA-specific Ig serum titer
OVA	181 (100)	1:1.373 (100)	1:110.2
OVA + AFL (mg/kg)			
200	22.5 ± 6.1* (12)	1:588* (47)	-
400	35.2 ± 4.3* (19)	1:432* (31)	-

[a] Groups of mice (n=10) were treated by intraperitoneal injection of the indicated doses of AFL (in mg/Kg/day) and additionally sensitized with OVA. Control animal group (OVA) received saline during the OVA sensitization. After the treatment period, animals were bled and the sera of each group were pooled. Total serum IgE concentration was determined by capture ELISA. OVA-specific IgE titers were measured by ELISA. The last column shows the titers of OVA-specific antibody (determined by the PCA test). The basal concentration of total IgE (5.10 ± 1.27 µg/ml) was obtained from animal that did not receive any treatment. Data are representative of three independent experiments.
* $p < 0.001$ compared with control group (OVA).
(-): Undetectable

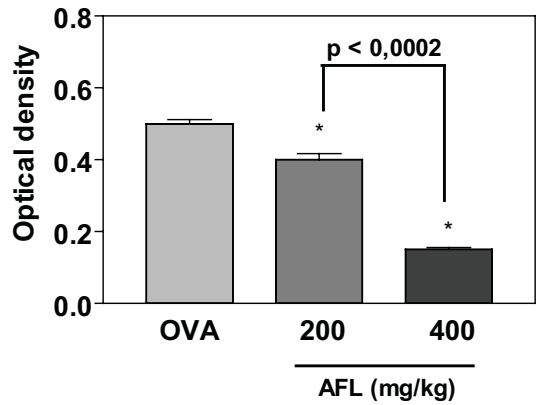

Figure 1. Effect of AFL on the background proliferative response. Groups of mice (n=10) were treated by intraperitoneal injection of the indicated doses of AFL (mg/kg/day) and additionally sensitized with OVA. Control animal group (OVA) received saline during the OVA sensitization. Spleen cells were obtained from each group of animals. Pooled spleen cells from each group were incubated at 5% CO_2, 37ºC for three days. The background cell proliferation was measured by the MTT test. Values are mean of optical density ± standard error of the mean (SEM) of triplicate cell cultures.
* $p < 0.001$ compared with control group (OVA).

related to lower total and OVA-specific serum IgE levels. Elevated IgE levels are associated with immediate-type allergic reactions (Mayr; Zuberi; Liu, 2003). Our data suggest that the intraperitoneal AFL administration may have an anti-allergic activity through the decrease of IgE production.

Th2 lymphocytes and its cytokines have a pivotal role in immediate allergic response (Packard; Khan, 2003). The inhibitory effect in the IgE production observed here could be for instance the result of an effect at the levels of cytokine production. Our previous results showed that spleen cells from normal BALB/c mice stimulated *in vitro* with AFL and Concanavalin-A produced high levels of IL-10 (Piuvezam et al., 1999). In addition, Bezerra-Santos (2004) demonstrated spleen cells from orally treated-AFL mice produced higher levels of both IFN-γ and IL-10, when compared to control animals. The IL-10 production in acute inflammatory diseases represents a favorable prognosis due to its anti-inflammatory effect (Jae-Won et al., 2002).

This prophylactic effect of the AFL could be related to an inhibitory effect on in *vivo* response to OVA. Indeed we observed that the AFL reduced the background proliferative cell response. This effect could be by an inhibition on basal cytokine production. It has been demonstrated that AFL reduced spleen cell proliferative response induced by Concanavalin-A and this effect was caused by increased of the IL-10 levels (Piuvezam et al., 1999).

AFL did not protect mice to anaphylactic shock reaction induced by compound 48/80. It is well known that this compound is a potent mast cell degranulator (Seung-Heon et al., 2005) and the mechanism involved in this anaphylactic response is not receptor dependent unlike IgE mediated response (Ferry et al., 2002). Besides that, it has been described that mast cell degranulators such as compound 48/80 induce histamine release throughout a plasma membrane change (Kim et al., 2000). Our finds may suggest that AFL is acting directly in the IgE production.

The results presented here showed that intraperitoneal AFL treatment inhibited anaphylactic shock reaction, IgE production and cell proliferative response. These data highlight the important role of this plant in regulating the murine IgE production and suggest its regulatory role in cytokine production in an IgE-dependent hypersensitivity model.

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